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Impacts of sublethal toxicity of cypermethrin on histopathological changes in gill of *labeo rohita* (hamilton, 1822).

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ABSTRCT

The present investigation was designed to understand the sublethal effects of cypermethrin, an active pyrethroid, a widely used against an extensive range of pests in agriculture. In this study Rohu (*Labeo rohita*) was subjected to the treatment with two sublethal concentrations (0.30mg/l and 0.15mg/l) of cypermethrin for a total period 10, 20 and 30 days. The results obtained indicated significant stress on the experimental fish due to pesticide intoxication.The histopathological study of the gill revealed changes as necrosis,vaculor degeneration, fusion of gill, twisting and shortening of secondary gills lamellae , atrophy of primary and secondary lamellae.

Key words: Biomarkers, Cypermethrin, Pesticides, Necrosis

INTRODUCTION

Histology is a non-specific, relatively low-cost and valuable ecological risk assessment method. Histopathological biomarkers are closely related to other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular change in the affected organism. The mechanism of action of several xenobiotics could initiate the formation of a specific enzyme that causes changes in metabolism further leading to cellular intoxication and death, at a cellular level, whereas this manifests as necrosis, as a biomarker at tissue level as well as from chemical insult, lesions may arise from infectious diseases and parasites, provoking necrotic and degenerative alternations to which the

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organism responds with an inflammatory defensive reaction (Japamalai, 2017)). Histopathological biomarkers are very useful for examining the structure of vital organs (gills, kidney, and liver) when respiration, excretion, or detoxification process are affected by environmental contaminants (Blahova *et al.*, 2014). During the contaminant exposure, histopathological observations can give insights into the organism's health and responses towards the stressors and therefore have been widely used biomarkers both in the laboratory and field studies (Geurgieva and Stoyanova 2016).

According to Mariyadasu and Kusumakumar (2017), organisms inhabiting in aquatic environments are considered biological sensitive, due to their ability, to respond to changes that occur in the water. Changes, occurring specifically of biochemical, histological and physical alterations, in fish populations due to chemical stress, are manifestations resulting and can give a relatively rapid indication of how environmental conditions affect fish populations. Fish populations will either adapt to environmental changes, or may result in mortality in low concentrations. To manage healthy fish populations, it is necessary to identify and are early detectable warning signs of damage on cellular level, before physiological and behavioural process are affected and can be achieved as bio indicator through histological analysis. Histopathological studies have been conducted to help for establishment of causal relationships between contaminant and exposure and other various biological responses.

Histopathology is a microscopic examination of tissue in order to study the manifestation. It is a clear picture of cyto-architectural changes produced during the chemical intoxication and help in assessing the extent of any toxicant (pesticide) pollution in the ecosystem. Histopathological alterations due to lethal and sublethal effects of toxic compounds are very important for detail prediction of health status of fish and for future ecological impact. Histopathological alterations in different tissues of fishes exposed to various toxicants have been studies by number of workers (Pawar and Patil 2019).

Cypermethrin is one of the synthetic pyrethroids, initially synthesized in 1974, and marketed from 1977 onwards as a highly active insecticide, effective against a wide range of pests in agriculture, animal husbandry and domestic situations (Ravi Sekhar *et al*., 2009). The current commercial products were evolved from the natural pyrethrin, which possess high

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insecticidal potency, low mammalian toxicity and very short persistence. These are highly toxic to fish and some aquatic invertebrates. (Al-Ghanim, 2014). It may affect the CNS, CVS as well as skin and eye irritant of fish (Tiwari *et a*l., 2019). Among the several aspects of toxicity studies, the bioassay constitutes one of the most commonly used methods in aquatic environmental studies with suitable organisms. The necessity of determining the toxicity of substances to commercially aquatic forms at the lower level of the food chain has been useful and accepted for water quality management (Zahn *et al.,* 2019). The current investigation was undertaken to investigate the sublethal effect of cypermethrin on histopatholoical changes in gill of *Labeo rohita.*

MATERIALS AND METHODS

Healthy 6.5 ± 1.2 g live weight of *Labeo rohita* were selected from stock tanks and exposed to two sublethal concentrations in each pesticides namely cypermethrin [0.30 mg/l ($1/5^{th}$ 96 hr LC_{50}) and 0.15 mg/l ($1/10^{th}$ 96 hr LC_{50})] .These two concentrations were maintained for 10, 20 and 30 days. Fresh test media and toxicants were supplied daily. Care was taken to see that all the fish introduced to each aquarium tank (15 litre) were least disturbed throughout the experimental tenure. The unfed material when collected six hrs after supplying food, to prevent contamination owing to decay. Simultaneously a control group of 10 individual was maintained throughout the experimental period. After exposure of 10, 20 and 30 days fish were sacrificed to obtain the necessary tissues for histopathological studies. Gill is isolated from normal and experimental fish, Physiological saline solution (0.75%NaCl) was used to rinse and clean the tissue and fixed in aqueous Bouins solution for 48 hrs processed through graded series of alcohols cleared in xylene and embedded in paraffin wax. They are processed by double embedding technique. Sections were cut of 6µ (microns) thickness, stained with Ehrlich haematoxylin and Eosin (dissolved in 70% alcohol) (Humason, 1972) and were mounted in *Canada balsam.* The photographs at 200x magnification were taken with computer aided microscope (They were examined under light microscope (COSLAB) with camera attached (MDCE-5C) and were photographed at 40x resolution).

RESULT

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Acute toxicity: Table 1 shows the LC_{50} value of 96 hrs were (1.507 mg /l) calculated using Probit analysis used for two sublethal exposure concentrations of 0.30mg/l ($1/5^{th}$ 96 hr LC_{50}) and 0.15mg/l ($1/10^{th}$ 96 hrs LC_{50}).

HISTOPATHOLOGICAL STUDIES

Gill histopathology: In control groups, gill lamellae were arranged uniformly with inter lamellar space. The gill is composed of primary lamellae and secondary lamellae were observed (Plate 1).

Effect of cypermethrin on gill at 10 days Exposure

After 10 days exposure of high concentrations of cypermethrin (0.30 mg/l) on fish *Labeo rohita* showed very common histopathological abnormalities, such as dislocation of cells, curling of secondary lamellae, lamellar fusion, disturbance the tips of secondary lamellae, dilated vessel and swollen of secondary lamellae (Plate 2). Besides the numerous lamellar mucous cells produced on the primary lamellae. While the fish *Labeo rohita* treated to low concentrations of cypermethrin (0.15 mg/l) on the fish expressed many alterations are curling of secondary lamellae, atrophy of mucous layer, necrotic lamellae, dislocation of primary gill lamellae, hyperplasia and aggregation of lamellar cells (Plate 3).

Effect of cypermethrin on gill at 20 days Exposure

The 20 days exposure of high concentration of cypermethrin (0.30 mg/l) on the fish *Labeo rohita* exhibit more alterations such as proliferation of chloride cells, interruption of secondary lamellar, necrotic lamellar, gill lesions, dislocation of primary lamellae and atrophy of mucosal layer (Plate 2). At low concentrations of cypermethrin (0.15 mg/l) exposure on the fish *L.rohita* showed lamellar deformities. In addition disruption of tip of secondary lamellar, sloughing of lamellar epithelium and aggregation of mucus cells (Plate 3).

Effect of cypermethrin on gill at 30 days Exposure

At 30 days of exposure increased hyperplasia at lowest and highest (0.30mg/l and 0.15mg/l) concentrations of cypermethrin. The gills of fresh water fish *Labeo rohita* exhibit blood congestion, lamellar fusion, disturbances of secondary lamellae, atrophy of primary lamellae and decline the length of secondary lamellae, increased hyperplasia, necrosis of lamellae,

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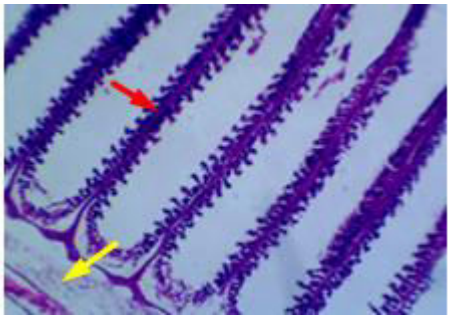
deterioration of primary lamellae, lamellar swollen, rupture of blood vessel and dislocation the

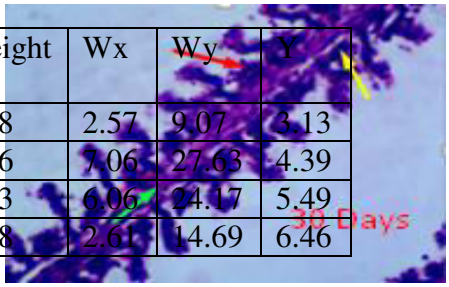


tip of secondary lamellae. In all concentrations of cypermethrin at 30 days the exposure, lamellar deformities were mainly noticed in this period (Plate 2 and 3).

of

Table 1 Log-Dose /Probit regression line analysis the response of *Labeo rohita* exposed to cypermethrin for 96 hrs

Dose (mg/l)	No	Mor %	Log Dose	Emp. Pro	Exp. Pro	Work Pro	Wt Coef	Weight w	Wx	Wy	Y
1.20	10	10.00	1.08	3.72	3.39	3.81	0.24	2.38	2.57	9.07	3.13
1.40	10	30.00	1.15	4.48	4.66	4.49	0.62	6.16	7.06	27.63	4.39
1.60	10	50.00	1.20	5.00	5.76	4.81	0.50	5.03	6.06	24.17	5.49
1.80	10	100.00	1.26	7.33	6.73	7.06	0.21	2.08	2.61	14.69	6.46



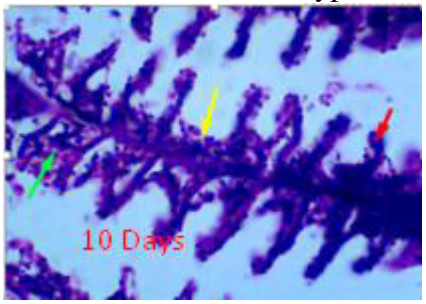
STATISTICS

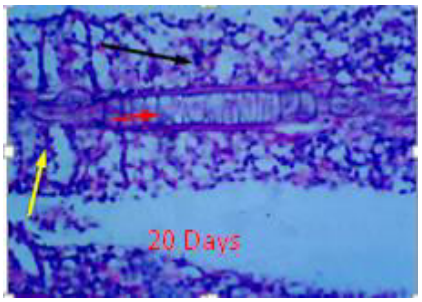
SW =15.650 SWX =18.296 X Bar =1.169 SWY =75.568 Y Bar =4.829
SWX*X =21.433 SWY*Y =378.466 SWXY =89.006 b value =18.946
Regression Equation Y =18.946 x -17.32 If Y =5.0 then X =1.178
This corresponds to dose of 1.507 Variance 0.0003 Chi-square 3.63 (with 2 Deg. Of freedom P) Lower Limit 1.1447 Log Dose 1.1781 Upper Limit 1.2116
LCL = 0.139 UCL = 0.162

Plate 1: Section of gill of control fish *Labeo rohita* showing normal structure

Yellow arrow-Primary lamellae
Red arrow-Secondary lamellae

Plate 2: Section of gill of fish *Labeo rohita* exposed to 1/5th sub lethal concentration of cypermethrin (0.30mg/l) for 10, 20 and 30 days





Red arrow-Curling of secondary lamellae Black arrow –Necrotic lamellae Green arrow –Blood congestion
Yellow arrow –Dislocation of cells Yellow arrow –Interruption of sec. lamellae Yellow arrow –Lamellar fusion
Red arrow –Proliferation of chloride cells Red arrow –Disturbance of sec. lamellae

Plate 3: Section of gill of fish *Labeo rohita* exposed to 1/10th sub lethal concentration of cypermethrin (0.15 mg/l) for 10, 20 and 30 days

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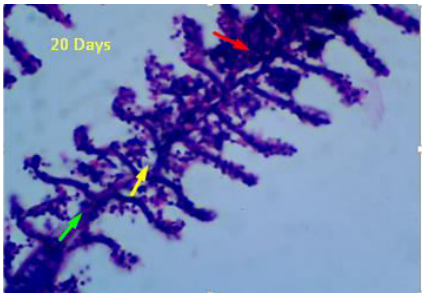
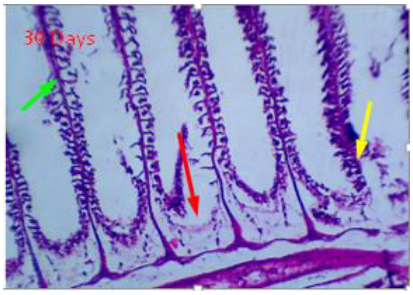
Blue arrow –Necrotic lamellae
Green arrow –Curling of sec. lamellae
Red arrow – Atrophy of mucous layer



Green arrow –Destruction of vessel
Yellow arrow –Disruption of pri. lamellae
Red arrow –Smooth muscle hypertrophy



Green arrow –Dying of lamellae
Yellow arrow –Atrophy of sec. lamellae
Red arrow –Increased hyperplasia

DISCUSSION

Gill Histopathology

In fish , gills are the vital organs, which serve as an utmost surface area in contact with the external environmental system accept dissolved toxicants from the aquatic environment. Gills play a vital role in respiration, osmoregulation, and excretion of nitrogenous waste products. The constant intake of pesticides through water which disturbs the chloride cells of gills in the aquatic organisms and also leads to affect the respiratory pigment to transport dissolved oxygen in the water and prolonged treated leads to anorexia condition.

The several histopathological alterations in the gills of *Labeo rohita* were noticed in the present study such as smooth muscle hypertrophy, sloughing of lamellar epithelium, lamellar fusion, disruption of blood vessel disturbance the tip of secondary lamellae, proliferation of chloride cells, hyperplasia, atrophy in a mucosal layer, curling of secondary lamellae in exposure of cypermethrin for 10,20 and 30 days. Similar findings have been reported by several authors using different pesticides and different fish species, which are following evidences.

Various histopathological studies indicated that gills are the first target organs of fishes on exposure to pesticides and are prime indicators to show water quality (Qureshi *et al*., 2016). Katuli *et al.,* (2014) reported that the impact of diazinon leads to severe necrotic lamellae in gill tissues of *Rutilus rutilus* and *Scophthalmus maximus.* Ghasemzadek *et al.,* (2015) found diazinon exposure in gills of Scatophag us argus exhibited Oedema, epithelial

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lifting, curling of secondary lamellae. Zahran *et al.,* (2018) noticed histopathological changes in the gills of *Oreochromis niloticus* on treated to two different dose of chlorpyrifos, resulted damage in the secondary lamellae, Mucus accumulation and severity at the tip of the primary lamellae.

Karmaker *et al.,* (2015) observed fusion of primary and secondary lamellae, epithelial hyperplasia and decrease of inter lamellar cell mass with increasing malathion concentration in gills of fish *Labeo rohita.* Selvi and Ilavazhahan (2012) studied the histological sections of gills in the fingerlings of *Catla Catla* treated with methyl parathion and reported alternations such as fusion of secondary lamellae as a result of hyperplasia, swelling of the epithelia cells and observance of mucoid metaplasia distinctly. Shah and Parveen (2022) reported that dilated vessel, lamellar epithelium in fish Gill tissue of *Cyprinus carpio* exposed to pesticides.

Akter *et al.,* (2020) observed the histopathological changes included hyperplasia, curling of secondary lamellae, haemorrhage, clubbing and necrosis in fish *Heteropneustes fossils* exposed to Envoy 50 sc. Kenthao *et al.*, (2020) investigated Histopathological appearances of gill after they were exposed with cypermethrin for 96 hr were desquamation, Oedema, hyperplasia and fusion of secondary lamellae. In addition there are accumulation of blood cells and a decrease of gill surface area.

Veeraiah *et al.,* (2015) studied that congestion and swelling occurs in gill lamellar epithelium, necrosis of primary lamellae and clubbing of the ends of the some secondary lamellae in fish *Labeo rohita* exposure of chlorpyrifos. Abdul Kareem *et al.*, (2020) reported various histopathological lesions recorded in the gills of *Hetero clarias* exposed to chlorpyrifos could probably be attributed to the oxidative stress induced by chlorpyrifos. Saleem Ahamad *et al.,* (2018) observed that fusion of secondary gills lamellae, lamellar disorganization and atrophy of lamellar region in fish rohu (*Labeo rohita*) exposed to cypermethrin. Bantu *et al.,* (2017) noticed gills beside absorbing oxygen perform many important functions such as regulation of ions, acid base balance and elimination of ecological toxicants affect these vital organs impose significant effects on health of fish.

CONCLUSION

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This study unravelled the toxic effects of commercial cypermethrin formulation on histopathological changes of gill in *Labeo rohita.* In this study suggested that cypermethrin significantly affected the histopathology like lamellar disorder, blood congestion, necrosis, haemorrhages, and atrophy of primary and secondary lamellae in gill, respectively. The effects are on the vital organ of gill, the possibility of the survival is limited and hence might be the causative factor of toxicity.

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